FORM PTO-1390 (REV. 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER	
SF0977X	
U.S. APPLICATION NO. (If known, see 37 CFR 1.5)	
09/869388	

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/30004	29 December 1999	31 December 1998

TITLE OF INVENTION

MONOCYTE-DERIVED NUCLEIC ACIDS AND RELATED COMPOSITIONS AND METHODS

APPLICANT(S) FOR DO/EO/US

BATES, Elizabeth; FOURNIER, Nathalie; CHALUS, Lionel and GARRONE, Pierre

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☐ has been communicated by the International Bureau.
   c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

Copy of IPER -International Preliminary Examination Report

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
	PCT/US99/30004	SF0977X

21. ☐ The following fees are submitted:

	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$690.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	16 - 20 =		x **$18.00**	$	
Independent claims	10 - 3 =	7	x **$80.00**	$ 560.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$270.00**	$	
TOTAL OF ABOVE CALCULATIONS =				$	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2. +				$	
SUBTOTAL =				$ 1,420.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$	
TOTAL FEES ENCLOSED =				$ 1,420.00	
				Amount to be refunded:	$
				charged:	$

a. ☐ A check in the amount of $ _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. 19-0365 in the amount of $ 1,420.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 19-0365. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Jaye P. McLaughlin
Patent Dept. K-6-1, 1990
Schering-Plough Corporation
2000 Galloping Hill Road
Kenilworth, New Jersey 07033-0530

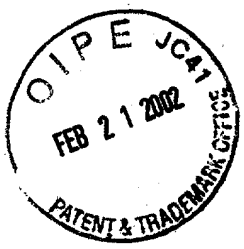
SIGNATURE

Jaye P. McLaughlin
NAME

41211
REGISTRATION NUMBER

June 28, 2001
Express Mail Label No. EL 664530719 US

PLEASE CHARGE ANY DEFICIENCY UP TO $300.00 OR CREDIT ANY EXCESS IN THE FEES DUE WITH THIS DOCUMENT TO OUR DEPOSIT ACCOUNT NO. 19-0365



File No. SF0977 X US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Elizabeth Bates *et al.*

Serial No.: 09/869,388 Group Art Unit: TBA

Filed: December 29, 1999 Examiner: TBA

Confirmation No.: 4303

For: MONOCYTE DERIVED NUCLEIC ACIDS AND RELATED COMPOSITIONS AND METHODS

PRELIMINARY AMENDMENT AND STATEMENT UNDER RULE 821

Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

Please consider the following in response to the NOTIFICATOIN OF DEFECTIVE RESPONSE mailed January 3, 2002 in this Application. Also enclosed is a Substitute Sequence Listing in paper and computer readable format along with an Associate Power of Attorney.

AMENDMENT

After the claims, please insert the following text on a separate page:

- -**Abstract of the Disclosure**

Nucleic acids encoding various monocyte-derived proteins and related compositions, including purified proteins and specific antibodies are described. Methods of using such composition are also provided.- -

Please delete the Sequence Listing and insert the enclosed Substitute Sequence Listing on separate pages after the "Abstract of the Disclosure"

REMARKS

Entry of the foregoing Amendment before substantive examination of the claims is requested. The Amendment enters a Substitute Sequence Listing and an Abstract of the Disclosure to the specification.

The sequences in the present, Substitute Sequence Listing were submitted with the original specification and the Abstract of the Disclosure appears in the corresponding International Application (PCT/US99/30004); accordingly, no new matter has been added to the Application.

Statement Under Rule 821

A diskette is enclosed which includes a Substitute Sequence Listing which corrects the errors indicated in the Notice. A paper copy of the file is attached.

The content of the attached paper entitled "SEQUENCE LISTING" and the accompanying, identically labeled diskette, specifically the text file therein labeled "seqlist.txt", is the same.

Early and favorable action is earnestly solicited.

Respectfully submitted,

Thomas Triolo, Ph.D.
Registration No. 48,001
Agent for Applicant(s)

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Bates *et al.*	:	Examiner: To Be Assigned
For: Monocyte-Derived Nucleic Acids and Related Compositions and Methods	:	Group Art Unit: To be Assigned
Serial No.: 09/869,388	:	
Filing Date: June 28, 2001	:	

Assistant Commissioner for Patents
Washington, D.C. 20231

AMENDMENT AND RESPONSE TO NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

This is in response to a Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures dated August 6, 2001. A response is due on or before October 6, 2001. Enclosed is a copy of the Notice, an Initial Sequence Listing (paper and computer readable format) and a Statement Under 37 C.F.R. § 1.821.

AMENDMENT

Please insert the enclosed Initial Sequence Listing at the end of the specification.

REMARKS

The content of the attached paper entitled "SEQUENCE LISTING" and of the accompanying, identically labeled diskette, specifically the text file therein labeled "seqlist.txt" is the same. Furthermore, the information in the attached "SEQUENCE LISTING" and in the text file contains no new matter.

CONCLUSION

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231 on this 4th day of October, 2001.

October 4, 2001
Date

Signature

Jaye McLaughlin
Reg. No. 41,211

Applicants believe that the present submission is fully responsive to the Notice. Early and favorable action is earnestly solicited.

Please charge any additional fees or credit overpayment to Deposit Account No. 19-0365.

Respectfully submitted,

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09/869388
SF0977X
JC18 Rec'd PCT/PTO 2 8 JUN 2001

MONOCYTE-DERIVED NUCLEIC ACIDS AND RELATED COMPOSITIONS AND METHODS

Field Of The Invention

The present invention is directed to compositions related to genes found in monocytes, cells which function in the immune system. These genes function in controlling development, differentiation, and/or physiology of the mammalian immune system. In particular, the invention provides nucleic acids, proteins, antibodies, and methods of using them.

Background Of The Invention

Monocytes are phagocytic cells that belong to the mononuclear phagocyte system and reside in the circulation. These cells originate in the bone marrow and remain only a short time in the marrow compartment once they differentiate. They then enter the circulation and can remain there for a relatively long period of time, e.g., a few days. Monocytes can enter the tissues and body cavities by a process known as diapedesis, where they differentiate into macrophages and possibly into dendritic cells. In an inflammatory response, the number of monocytes in the circulation may double, and many of the increased number of monocytes diapedese to the site of inflammation. For a review of monocytes and their functions, see, e.g., Gallin, *et al.* (eds), 1988, *Inflammation: Basic Principles and Clinical Correlates*, Raven Press, NY; van Furth (ed), 1985, *Mononuclear Phagocytes: Characteristics, Physiology and Function*, Martinus Nijhoff, Dordrecht, Netherlands.

Antigen presentation refers to the cellular events in which a proteinaceous antigen is taken up, processed by antigen presenting cells (APC), and then recognized to initiate an immune response. The most active antigen presenting cells have been characterized as the macrophages, which are direct developmental products from monocytes; dendritic cells; and certain B cells.

Macrophages are found in most tissues and are highly active in internalization of a wide variety of protein antigens and microorganisms. They have a highly developed endocytic activity, and secrete many products important in the initiation of an immune response. For this reason, it is believed that many genes expressed by monocytes or induced by monocyte activation are important in antigen uptake, processing, presentation, or regulation of the resulting immune response.

Despite the importance of monocytes to immune system function, these cells remain poorly characterized, both in terms of the proteins they express and in terms of many of their functions, in particular, the processes and mechanisms related to the initiation of an immune response, including antigen processing and presentation. There is thus a need in the art for agents useful in the diagnosis and treatment of medical conditions caused by, e.g., the inappropriate regulation, development, or physiology of antigen presenting cells.

Summary Of The Invention

The present invention fulfills this need by providing compositions and methods for determining the presence, amount, distribution and normalcy of certain gene products and for facilitating the discovery of agents for treating certain disease states.

The invention is based upon the discovery of novel genes and gene products isolated from activated monocytes.

The invention provides isolated nucleic acid sequences comprising at least about 12, preferably at least about 18, most preferably at least about 20-35, and most preferably 35-55 or more consecutive nucleotides shown in SEQ ID NO: 1, 3, 5, 7, or 9, or which encode an amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8 or 10, including complete protein coding sequences, and complements thereof. The invention encompasses sequence-conservative variants and function-conservative variants of these sequences. The nucleic acids may be DNA, RNA, DNA/RNA duplexes, protein-nucleic acid (PNA), or derivatives thereof. The invention also encompasses recombinant DNA vectors (including DNA expression vectors) comprising these sequences; cells comprising such vectors, including bacterial, fungal, plant, insect, and mammalian cells; and methods for producing expression products comprising RNA and polypeptides encoded by the sequences.

Polypeptide sequences of the invention comprise at least eight, preferably at least about 10, and more preferably at least about 12 or more consecutive amino acid residues derived from SEQ ID NO: 2, 4, 6, 8 or 10. Function-conservative variants and homologs are included in the scope of the invention.

The invention further provides binding compositions, in particular antibodies, most particularly monoclonal antibodies, which specifically bind to polypeptides having an amino acid sequence shown in SEQ ID NO: 2, 4, 6, 8 or 10 or function conserved variants or homologs thereof. Methods are also provided for producing antibodies having the desired binding specificity in a host animal.

Detailed Description Of The Invention

All patent applications, patents, and literature references cited in this specification are hereby incorporated herein by reference in their entirety.

In practicing the present invention, many conventional techniques in molecular biology, microbiology, and recombinant DNA, are used. Such techniques are well known and are explained fully in, for example, Sambrook *et al.*, 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; *DNA Cloning: A Practical Approach*, Volumes I and II, 1985 (D.N. Glover ed.); *Oligonucleotide Synthesis*, 1984, (M.L. Gait ed.); *Nucleic Acid Hybridization*, 1985, (Hames and Higgins); *Transcription and Translation*, 1984 (Hames and Higgins eds.); Animal Cell Culture, 1986 (R.I. Freshney ed.); *Immobilized Cells and Enzymes*, 1986 (IRL Press); Perbal, 1984, *A Practical Guide to Molecular Cloning*; the series, *Methods in Enzymology* (Academic Press, Inc.); *Gene Transfer Vectors for Mammalian Cells*, 1987 (J. H. Miller and M. P. Calos eds., Cold Spring Harbor Laboratory); and *Methods in Enzymology* Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively).

Definitions

1. A "monocyte-derived" nucleic acid or polypeptide refers to the source from which the sequence was originally isolated.

2. "Nucleic acid" or "polynucleotide" refers to purine- and pyrimidine-containing polymers of any length, either polyribonucleotides or polydeoxyribonucleotides or mixed polyribo-polydeoxyribo nucleotides. This includes single- and double-stranded molecules, i.e., DNA-DNA, DNA-RNA and RNA-RNA hybrids, as well as "protein nucleic acids" (PNA) formed by conjugating bases to an amino acid backbone. This also includes nucleic acids containing modified bases.

3. A "coding sequence" or a "protein-coding sequence" is a polynucleotide sequence capable of being transcribed into mRNA and/or capable of being translated into a polypeptide. The boundaries of the coding sequence are typically determined by a translation start codon at the 5'-terminus and a translation stop codon at the 3'-terminus.

4. A "complement" of a nucleic acid sequence refers to the "antisense" sequence that participates in Watson-Crick base-pairing with the original sequence.

5. An "isolated" nucleic acid or polypeptide refers to component that is removed from its original environment (for example, its natural environment if it is

naturally occurring). An isolated nucleic acid or polypeptide preferably contains less than about 50%, more preferably less than about 75%, and most preferably less than about 90%, of the cellular components with which it was originally associated.

6. A nucleic acid or polypeptide sequence that is "derived from" a designated sequence refers to a sequence that corresponds to a region of the designated sequence. For nucleic acid sequences, this encompasses sequences that are homologous or complementary to the sequence, as well as "sequence-conservative variants" and "function-conservative variants." For polypeptide sequences, this encompasses "function-conservative variants." Sequence-conservative variants are those in which a change of one or more nucleotides in a given codon position results in no alteration in the amino acid encoded at that position. Function-conservative variants are those in which a given amino acid residue in a polypeptide has been changed without substantially altering the overall conformation and function of the native polypeptide, including, but not limited to, replacement of an amino acid with one having similar physico-chemical properties (such as, for example, acidic, basic, hydrophobic, and the like). "Function-conservative" variants also include any polypeptides that have the ability to elicit antibodies specific to a designated polypeptide.

7. A "probe" refers to a nucleic acid or oligonucleotide that forms a hybrid structure with a sequence in a target region due to complementarity of at least one sequence in the probe with a sequence in the target.

8. Nucleic acids are "hybridizable" to each other when at least one strand of nucleic acid can anneal to another nucleic acid strand under defined stringency conditions. Stringency of hybridization is determined, e.g., by a) the temperature at which hybridization and/or washing is performed, and b) the ionic strength and polarity (e.g., formamide) of the hybridization and washing solutions, as well as other parameters. Hybridization requires that the two nucleic acids contain substantially complementary sequences; depending on the stringency of hybridization, however, mismatches may be tolerated. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementarity, variables well known in the art.

9. An "immunogenic component" is a moiety that is capable of eliciting a humoral and/or cellular immune response in a host animal.

10. An "antigenic component" is a moiety that binds to its specific antibody with sufficiently high affinity to form a detectable antigen-antibody complex.

11. A "sample" refers to a biological sample, such as, for example, tissue or fluid isolated from an individual or from an *in vitro* cell culture constituents, as well as samples obtained from laboratory procedures.

The invention provides nucleic acid sequences encoding mammalian proteins expressed on monocytes. While specific human monocyte-derived genes and gene products are described herein, the invention encompasses structurally (e.g., sequence) related embodiments from other sources or mammalian species, including polymorphic or individual variants. These will include, e.g., proteins which exhibit relatively few changes in sequence, e.g., less than about 5%, and number, e.g., less than 20 residue substitutions, typically less than 15, preferably less than 10, and more preferably less than 5 substitutions. These will also include versions which are truncated from full length and fusion proteins containing substantial segments of these sequences.

A gene/gene product, isolated from human monocyte cell library and designated FDF03, has been previously described in published International application WO 98/24906, the disclosure of which is incorporated herein in its entirety by reference. The FDF03 gene encodes a type I transmembrane protein comprising an extracellular portion characterized by Ig-like domains, indicating that this gene encodes a receptor member of the Ig superfamily.

SEQ NO: 1 shows the nucleic acid sequence encoding human FDF03 protein. The amino acid sequence of the FDF03 protein is shown in SEQ ID NO: 2. The putative coding region runs from about nucleotide 154 to nucleotide 1062. An N-terminal hydrophobic sequence corresponding to a putaive signal sequence runs from about amino acid residue -19 (Met) to amino acid residue -1 (Leu). An internal hydrophobic sequence corresponding to a putative transmembrane segment runs from about residue 177 (Ala) to residue 199 (Leu). The extracellular region is about 170 amino acids, with a potential Ig-like domain structure. The intracellular region is about 80 residues. Sequence analysis indicates similarity to GenBank clones H26010 and R50327 from humans.

Four human FDF03 homologs have now been discovered.

FDF03-ΔTM

The second human clone, designated FDF03-deltaTM (FDF03-ΔTM), appears to be a soluble form of human FDF03 generated by alternative splicing. The nucleic acid sequence encoding FDF03-ΔTM is shown in SEQ NO: 3. The amino acid sequence of the FDF03-ΔTM protein is shown in SEQ ID NO: 4.

cDNA of the FDF03-ΔTM molecule was amplified along with that of FDF03 during the analysis of human FDF03 expression by RT-PCR. Using primers designed in the 5'-UTR and 3'-UTR of FDF03 gene (FDF03-U25: 5'-ACAGCCCTCTTC-GGAGCCTCA (SEQ ID NO: 11) and FDF03-L1166: 5'-AAGCTGGCCCTGAACT CCTGG (SEQ ID NO: 12)), an approximately 200 base pair shorter band was amplified by RT-PCR from PMA/ionomycin activated PBL cDNA, then gel purified, cloned and sequenced. Different clones contained an identical insert of 943 base pairs with an open reading frame encoding a type I protein of 230 amino acids. The deduced amino acid sequence of FDF03-ΔTM matched perfectly with that of FDF03, but contained a gap of 73 amino acids that deleted the extracellular threonine-rich region and the transmembrane domain of FDF03. This resulted in a protein with a potential hydrophobic signal peptide followed by the extracellular Ig like-domain linked to the intracytoplasmic domain of FDF03. cDNA alignments with FDF03 sequence identified a deletion of 219 nucleotides in the FDF03-ΔTM sequence (FDF03 nucleotide 608 to 827) that did not introduce premature stop codons, suggesting that this molecule is the product of an alternative splicing. This molecule is believed to be a secreted soluble form of FDF03 and believed to bind to the same ligand(s) as FDF03.

The protein alignment of the FDF03 (SEQ ID NO: 2) and FDF03-ΔTM (SEQ ID NO: 4) is shown below.

```
1   MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE   FDF03
1   MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE   FDF03-ΔTM

61  LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQ   FDF03
61  LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQ   FDF03-ΔTM

121 SVYFCRVELDTRSSGRQQWQSIEGTKLSITQAVTTTTQRPSSMTTTWRLSSTTTTTGLRV   FDF03
121 SVYFCRVELDTRSSGRQQWQSIEGTKLSITQ----------------------------   FDF03-ΔTM

181 TQGKRRSDSWHISLETAVGVAVAVTVLGIMILGLICLLRWRRRKGQQRTKATTPAREPFQ   FDF03
152 ----------------------------------------GQQRTKATTPAREPFQ   FDF03-ΔTM

241 NTEEPYENIRNEGQNTDPKLNPKDDGIVYASLALSSSTSPRAPPSHRPLKSPQNETLYSV   FDF03
168 NTEEPYENIRNEGQNTDPKLNPKDDGIVYASLALSSSTSPRAPPSHRPLKSPQNETLYSV   FDF03-ΔTM

303 LKA                                                             FDF03
230 LKA                                                             FDF03-ΔTM
```

(- : deletion)

FDF03-S1

The third clone, designated FDF03-Short1 (FDF03-S1), is an Ig-like molecule homologous to FDF03 but with a short intracytoplasmic domain and a charged residue in

transmembrane domain. Comparative DNA and protein analysis suggests the presence of different genes for FDF03 and FDF03S1, rather than alternatively spliced products. The nucleic acid sequence encoding FDF03-S1 is shown in SEQ NO: 5. The amino acid sequence of the FDF03-S1 protein is shown in SEQ ID NO: 6.

FDF03-S1 is a type I transmembrane protein belonging to the Ig superfamily. FDF03-S1 contains a hydrophobic leader sequence followed by an extracellular region (~170 residues) with a V-type Ig domain structure homologous to that of FDF03 (88% homology at the amino acid level). Unlike FDF03, FDF03-S1 possesses a transmembrane domain with a charged amino acid (K), and a small intracellular tail (15 residues) without ITIM or internalization motif. FDF03-S1 is believed to represent an activation isoform of FDF03 and may associate with ITIM-bearing molecules such as DAP12. The amino acid sequence is shown below, wherein the signal peptide and transmembrane domain are underlined. The charged amino acid, lysine (K), residue (arrow) in the transmembrane domain may permit association with another chain, for example DAP12.

MGRPLLLPLLLLLQPPAFLQPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWEL
AIVPNVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQESGFLRISNLRKEDQSV
YFCRVELDTRRSGRQQLQSIKGTKLTITQAVTTTTTWRPSSTTTIAGLRVTESKGHSESWH
LSLDTAIRVALAVAVLKTVILGLLCLLLLWWRRRKGSRAPSSDF(SEQ ID NO: 6)
↑

The protein alignment of FDF03 (SEQ ID NO: 2) and FDF03-S1 (SEQ ID NO: 6) is shown below.

```
1    MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE  FDF03
1    MGRPLLLPLLLLLQPPAFLQPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE  FDF03-S1
              +  +        +

61   LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQ  FDF03
61   LAIVPNVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQESGFLRISNLRKEDQ  FDF03-S1
       ++ +                                          +          + +

121  SVYFCRVELDTRSSGRQQWQSIEGTKLSITQAVTTTTQRPSSMTTTWRLSSTTTTTGLRV  FDF03
121  SVYFCRVELDTRRSGRQQLQSIKGTKLTITQAVTTTT........TWRPSSTTTIAGLRV  FDF03-S1
                 +      +    +     +           ++++++++    +      ++

181  TQGKRRSDSWHISLETAVGVAVAVTVLGIMILGLICLL..RWRRRKGQQRTKATTPAREP  FDF03
173  TESKGHSESWHLSLDTAIRVALAVAVLKTVILGLLCLLLLWWRRRKGSRAPSSDF       FDF03-S1
      ++ ++ +    +   +   ++   +   +   +++     +    +++       ++++++++

239  FQNTEEPYENIRNEGQNTDPKLNPKDDGIVYASLALSSSTSPRAPPSHRPLKSPQNETLY  FDF03

299  SVLKA                                                          FDF03

     + : residue different or gap
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Distribution studies (RT-PCR) shows strong expression in B cells (pool resting + activated), T cells and PBL. Lower expression was observed in monocytes, dendritic cells and granulocytes.

FDF03-M14

The fourth clone, desginated FDF03-M14, is a potential soluble form of human FDF03 generated by alternative splicing. The nucleic acid sequence encoding FDF03-M14 is shown in SEQ ID NO: 7. The amino acid sequence of the FDF03-M-14 protein is shown in SEQ ID NO: 8. cDNA of this molecule was amplified along with that of FDF03 during the analysis of human FDF03 expression by RT-PCR. Using primers designed in the 5'-UTR and 3'-UTR of FDF03 gene (FDF03-U25: 5'-ACAGCCC-TCTTCGGAGCCTCA (SEQ ID NO: 11) and FDF03-L1166: 5'-AGCTGGCCCTGA-ACTCCTGG (SEQ ID NO: 12)), an approximately 200 base pair shorter band was amplified by RT-PCR from activated PBL cDNA, then gel purified, cloned and sequenced. One clone (M14) contained an insert of 908 base pairs with an ORF encoding a type I protein of 175 amino acids. cDNA alignments with FDF03 sequence identified a deletion of 253 nucleotides in FDF03-M14 sequence (FDF03 nucleotide 608 to 861) that deleted the sequences encoding the extracellular threonine-rich region, the transmembrane domain and the start of the intracytoplasmic domain of FDF03, and that introduced a prematured stop codon at position 655 of FDF03-M14. The deduced amino acid sequence of FDF03-M14 resulted in a protein with a potential hydrophobic signal peptide followed by an extracellular Ig like-domain that matched perfectly with that of FDF03, but that was linked to a COOH-terminal 24 amino acid sequence different from FDF03. This molecule may be the product of an alternative splicing of FDF03 mRNA.

Like FDF03-ΔTM, this molecule may represent a secreted soluble form of FDF03 and may bind to the same ligand(s) as FDF03. The amino acid sequence is shown below, wherein the signal sequence is underlined.

MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWEL
ATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQSV
YFCRVELDTRSSGRQQWQSIEGTKLSITQGNPSKTQRSHMRISGMRDKIQIPS (SEQ ID
NO: 8)

The protein alignment of FDF03 (SEQ ID NO: 2) and FDF03-M14 (SEQ ID NO: 8) is shown below.

```
1    MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE     FDF03
1    MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE     FDF03-14

61   LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQ     FDF03
61   LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQ     FDF03-M14
```

```
121  SVYFCRVELDTRSSGRQQWQSIEGTKLSITQAVTTTTQRPSSMTTTWRLSSTTTTTGLRV      FDF03
121  SVYFCRVELDTRSSGRQQWQSIEGTKLSITQGNPSKTQRSHMRISGMRDKIQIPS           FDF03-M14
                                  *****   ******** *******

181  TQGKRRSDSWHISLETAVGVAVAVTVLGIMILGLICLLRWRRRKGQQRTKATTPAREPFQ      FDF03

241  NTEEPYENIRNEGQNTDPKLNPKDDGIVYASLALSSSTSPRAPPSHRPLKSPQNETLYSVLKA  FDF03

*: residue different
```

FDF03-S2

The fifth clone, designated FDF03-S2 is an Ig-like molecule homologous to FDF03 but with a short intracytoplasmic domain and a charged residue in transmembrane domain. This molecule is highly homologous to FDF03-S1 and is a potential DAP12-associated protein. The nucleic acid sequence encoding FDF03-S2 is shown is SEQ ID NO: 9. The amino acid sequence of the FDF03-S2 protein is shown in SEQ ID NO: 10.

cDNA of this molecule was amplified using primers specific for FDF03-S2. Specificity is obtained with forward primer designed in 5'UTR of FDF03-S2. FDF03-S2-forward: 5'-CAAGG- GATAAAAAGGCAC (SEQ ID NO: 13) (does not amplify FDF03, FDF03ΔTM or FDF03-S1). FDF03-S2-reverse: 5'-AACTCTCCTCCAGTCGGT (SEQ ID NO: 14) (can amplify FDF03-S1, but not FDF03 or FDF03deltaTM).

FDF03-S2 is a type I transmembrane protein belonging to the Ig superfamily. FDF03-S2 contains a hydrophobic leader sequence followed by an extracellular region (~170 residues) with one V-type Ig domain structure homologous to that of FDF03 (~85% homology at the amino acid level). Unlike FDF03, FDF03-S2 possesses a transmembrane domain with a charged amino acid (K), and a small intracellular tail (15 residues) without ITIM or internalization motif. FDF03-S2 is highly homologous to FDF03-S1 (3 amino acid difference in the extracellular domain and one amino acid missing in the transmembrane domain). Like FDF03-S1, FDF03-S2 may represent an activation isoform of FDF03 and may associate with ITAM-bearing molecules such as DAP12.

There are two putative start codons in frame (position 117 and 309). The first one is not contained within a typical Kozak sequence. The sequence shown below is deduced from the second start codon (nucleotide 309), as starting at the first start codon in frame (position 117) does not encode for a hydrophobic sequence followed by another Ig-like domain. In the sequence shown below, the signal peptide and transmembrane domain are underlined. The charged amino acid, lysine (K) residue (arrow) in transmembrane domain may permit association with another chain, for example DAP12.

MGRPLLLPLLLLLLQPPAFLQPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE
LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQESGFLRISNLRKEDQ
SVYFCRVELDTRRSGRQQLQSIKGTKLTITQAVTTTTTWRPSSTTTIAGLRVTESKGHSE
SWHLSLDTAIRVALAVAVLKTVILGLLCLLLWWRRRKGSRAPSSDF
↑

The protein alignments of FDF03, FDF03-S1 and FDF03-S2 is shown below.

```
1    MGRPLLLPLLPLLLPPAFLQPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE  FDF03
1    MGRPLLLPLLLLLQPPAFLQPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE  FDF03-S1
1    MGRPLLLPLLLLLQPPAFLQPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWE  FDF03-S2
              +  +        +

61   LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGFLRISNLQKQDQ  FDF03
61   LAIVPNVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQESGFLRISNLRKEDQ  FDF03-S1
61   LATAPDVRISWRRGHFHGQSFYSTRPPSIHKDYVNRLFLNWTEGQESGFLRISNLRKEDQ  FDF03-S2
** *                                          +          + +

121  SVYFCRVELDTRSSGRQQWQSIEGTKLSITQAVTTTTQRPSSMTTTWRLSSTTTTTGLRV  FDF03
121  SVYFCRVELDTRRSGRQQLQSIKGTKLTITQAVTTTT........TWRPSSTTTIAGLRV  FDF03-S1
121  SVYFCRVELDTRRSGRQQLQSIKGTKLTITQAVTTTT........TWRPSSTTTIAGLRV  FDF03-S2
                 +     +   +    +          ++++++++   +      ++

181  TQGKRRSDSWHISLETAVGVAVAVTVLGIMILGLICLL..RWRRRKGQQRTKATTPAREP  FDF03
173  TESKGHSESWHLSLDTAIRVALAVAVLKTVILGLLCLLLLWWRRRKGSRAPSSDF       FDF03-S1
173  TESKGHSESWHLSLDTAIRVALAVAVLKTVILGLLCLLL.WWRRRKGSRAPSSDF       FDF03-S2
      ++ ++ +   +  +  ++  +  +  +++    +   +*+      ++++++++

239  FQNTEEPYENIRNEGQNTDPKLNPKDDGIVYASLALSSSTSPRAPPSHRPLKSPQNETLY  FDF03

299  SVLKA                                                          FDF03

+ : residue different or gap between FDF03-S2/FDF03-S1 and FDF03
* : residue different or gap between FDF03-S2/FDF03 and FDF03-S1
```

Distribution studies (RT-PCR) shows expression in activated dendritic cells (CD34-derived), PBMC, monocytes and tonsil B cells.

Alignment with human IgV domains and TCR V domain is given below. This alignment shows the conserved VDJ structure of FDF03.

```
Ig V region QVQ.LQESGPG.LVKPSETLSLTCTVSGGSVSSGSYYWSW.IRQAPGKGLEWIG
TCR human   QVQ.LQESGPG.LVKPSETLSLTCTVSGYSISSG.YYWGW.IRQPPGKGLEWIG
FDF03       QPSGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWELATAPDVRISWRR
FDF03-S1     QPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWELAIVPNVRISWRR
FDF03-S2     QPGGSTGSGPSYLYGVTQPKHLSASMGGSVEIPFSFYYPWELATAPDVRISWRR
            +      +++  +        +     +        +  +     +     +

Ig V region YIYYSGSTNY.......NRSHKSRVNIS.VDTAKNQFSLKLSSVSTADTAVYYCARIT
TCR human   SIYHSGSTYY.......NPSLKSRVTIS.VDTSKNQFSLKLSSVTAADTAVYYCARVR
FDF03       GHFH.GQSFYSTRPPSIHKDYVNRLFLNWTEGQKSGF.LRISNLQKQDQSVYFC.RVE
FDF03-S1     GHFH.GQSFYSTRPPSIHKDYVNRLFLNWTEGQESGF.LRISNLRKEDQSVYFC.RVE
FDF03-S2     GHFH.GQSFYSTRPPSIHKDYVNRLFLNWTEGQESGF.LRISNLRKEDQSVYFC.RVE
                 +   +             +            + +  +     +  ++ + +


Ig V region TTVPSSWYYYYMDVWDKGTTVTVSS
TCR human   RRYSSSAS...KIIFGSGTRLSIR.
FDF03       LDTRSSGRQQWQS..IEGTKLSITQ
FDF03-S1     LDTRRSGRQQLQS..IKGTKLTITQ
FDF03-S2     LDTRRSGRQQLQS..IKGTKLTITQ
                 +          ++
```

Studies of human genomic DNA clones show that chromosome 7 contains both FDF03-S1 and FDF03 specific sequences, confirming that the two molecules are encoded by two different genes. These studies also suggest that FDF03-S1 and -S2 genes are two different alleles of the same gene. In addition, PCR from intronic sequence surrounding the areas of difference between S1 and S2 on genomic DNA from different donors shows the existence of homozygotes and S1/S2 heterozygotes at this locus. It is thus likely that these two cDNAs are from different alleles.

The genomic organization of the FDF03 gene confirms that FDF03-ΔTM is produced by alternative splicing (deletion of exon 3 coding for the hinge region and TM domain). This is also the case for FDF03-M14 (deletion of exons 3 and 4).

The two forms of FDF03-S1/2 may be advantageously used as population markers. The two forms of this protein will either not bind the same ligand (e.g., as in the case of the NK receptor family) or will bind at different affinities, thus potentially giving individuals a different response to receptor/ligand interaction.

The localization of the genes encoding FDF03 (including the ΔTM and M14 forms) and FDF03-S1 on human chromosome 7q22 is interesting because this region is frequently deleted in myelodystrophic syndromes such as Acute Myeloid Leukemia (AML). The implication of the possible deletion of a myeloid inhibitory receptor in a proliferative disease leads to a possibile use in gene therapy.

<u>Nucleic Acids, Vectors, and Host Cells</u>

The invention provides nucleic acid sequences, in particular the nucleic acid sequences shown in SEQ ID NO: 1, 5, 7 or 9 or nucleic acid sequences which encode an amino acid sequences shown in SEQ ID NO: 2, 4, 6, 8 or 10. The invention encompasses isolated nucleic acid fragments comprising all or part of the individual nucleic acid sequences disclosed herein. The nucleic acid sequences of the invention comprise at least about 12, preferably at least about 18, more preferably at least about 20-35 and most preferably at least about 35-55 or more consecutive nucleotides, including complete protein-coding sequences, or complements thereof. The invention encompasses sequence-conservative variants and function-conservative variants of these sequences.

Nucleic acids comprising any of the sequences disclosed herein or subsequences thereof can be prepared by standard methods using the nucleic acid sequence information provided in SEQ ID NO: 1, 3, 5, 7 and 9. For example, nucleic acids can be chemically synthesized using, e.g., the phosphoramidite solid support method of Matteucci *et al.*,

1981, *J. Am. Chem. Soc.* **103**:3185, the method of Yoo *et al.*, 1989, *J. Biol. Chem.* **764**:17078, or other well known methods. This can be done by sequentially linking a series of oligonucleotide cassettes comprising pairs of synthetic oligonucleotides. The nucleic acids may be isolated directly from cells. Alternatively, the polymerase chain reaction (PCR) method can be used to produce the nucleic acids of the invention, using either chemically synthesized strands or genomic material as templates. Primers used for PCR can be synthesized using the sequence information provided herein and can further be designed to introduce appropriate new restriction sites, if desirable, to facilitate incorporation into a given vector for recombinant expression. Of course, due to the degeneracy of the genetic code, many different nucleotide sequences can encode polypeptides having the amino acid sequences defined by SEQ ID NO: 2, 4, 6, 8 or 10 subsequences thereof. The codons can be selected for optimal expression in prokaryotic or eukaryotic systems. Such degenerate variants are also encompassed by this invention.

The encoded polypeptides may be expressed by using many known vectors such as pUC plasmids, pET plasmids (Novagen, Inc., Madison, WI), or pRSET or pREP (Invitrogen, San Diego, CA), and many appropriate host cells such as *Escherichia coli*, *Saccharomyces cerevisiae*, and insect and mammalian cell lines using methods known to those skilled in the art. The particular choice of vector/host is not critical to the practice of the invention.

The nucleic acids of the present invention find use, e.g., as templates for the recombinant production of peptides or polypeptides, as probes and primers for the detection of the human genes described herein, for chromosome mapping, and as probes or to design PCR primers to identify homologous genes in other mammalian species. Homology may be determined experimentally. Alternatively, homology analysis may be performed computationally. In practicing the present invention, a gene that shares at least about 70% DNA sequence homology at the nucleotide level with the genome of another mammalian species is considered to be present in that species. The determination that a gene is present in another mammal may be achieved using any technique known in the art. Appropriate techniques include without limitation hybridization to genomic DNA, colony hybridization to a genomic or cDNA library, polymerase chain reaction (PCR) using degenerate primers or gene-specific primers and genomic DNA as a template, genetic complementation, antibody cross-reactivity, or biochemical complementation *in vitro*.

In applying these techniques, conditions are established that discriminate different levels of homology between probe and template. For example, for hybridization of a

probe to immobilized DNA (whether in a Southern blot, dot blot, or colony hybridization format), varying the SSC concentration in the buffer allows the detection of hybrids having different levels of homology (1X SSC is 0.15 M NaCl - 0.015 M Na citrate). In a wash buffer containing 6M urea and 0.4% sodium dodecyl sulfate, the presence of 2X SSC, 0.5X SSC, 0.1X SSC, and 0.05X SSC allows the formation of hybrids having threshold homologies of at least 55% + 5%, 65% + 5%, 75% + 5%, and >85%, respectively. Preferably, once a gene has been identified in another organism by hybridization or PCR, the DNA sequence of the gene is determined directly.

It will be understood that some methods that detect homologous sequences may result in the identification or isolation of only a portion of the entire protein-coding sequence of a particular gene. The entire protein-coding sequence can be isolated and identified, for example, by using an isolated nucleic acid encoding the known portion of the sequence, or fragments thereof, to prime a sequencing reaction with cDNA as template; this is followed by sequencing the amplified product. The isolated nucleic acid encoding the disclosed sequence, or fragments thereof, can also be hybridized to appropriate cDNA libraries to identify clones containing additional complete segments of the protein-coding sequence of which the shorter sequence forms a part. Then, the entire protein-coding sequence, or fragments thereof, or nucleic acids encoding all or part of the sequence, or sequence-conservative or function-conservative variants thereof, may be employed in practicing the present invention.

In a similar manner, additional sequences derived from the 5' and 3' flanking regions of sequence encoding the protein, including regulatory sequences, may be isolated, and the nucleotide sequence determined.

Polypeptides

Both the naturally occurring and recombinant forms of the polypeptides described herein, including both glycosylated and non-glycosylated forms are encompassed by the invention. The polypeptides of the present invention, including function-conservative variants, may be isolated from human monocytes, or from heterologous organisms or cells (e.g., bacteria, fungi, insect, plant, and mammalian cells) into which a protein-coding sequence has been introduced and expressed. The proteins described herein, or portions thereof, also may be expressed as fusions with other proteins. The polypeptides may be chemically synthesized by commercially available automated procedures, including, without limitation, exclusive solid phase synthesis, partial solid phase

methods, fragment condensation or classical solution synthesis. The polypeptides can also, advantageously, be made by *in vitro* translation.

Methods for polypeptide purification are well-known in the art, including, without limitation, preparative disc-gel electrophoresis, isoelectric focusing, sucrose density gradient centrifugation, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. For some purposes, it is preferable to produce the polypeptide in a recombinant system in which the protein contains an additional sequence tag that facilitates purification, such as, but not limited to, a polyhistidine sequence. The polypeptide can then be purified from a crude lysate of the host cell by chromatography on an appropriate solid-phase matrix. Alternatively, antibodies produced against a protein or against peptides derived therefrom can be used as purification reagents. Other purification methods are possible.

The present invention also encompasses derivatives and homologues of the polypeptides specifically disclosed herein. For some purposes, nucleic acid sequences encoding the peptides may be altered by substitutions, additions, or deletions that provide for functionally equivalent molecules, i.e., function-conservative variants. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of similar properties, such as, for example, positively charged amino acids (arginine, lysine, and histidine); negatively charged amino acids (aspartate and glutamate); polar neutral amino acids; and non-polar amino acids.

The isolated polypeptides may be modified by, for example, phosphorylation, sulfation, acylation, or other protein modifications. They may also be modified with a label capable of providing a detectable signal, either directly or indirectly, including, but not limited to, radioisotopes and fluorescent compounds.

The polypeptides of the invention find use, e.g., for binding studies, for construction and expression of modified molecules, for structure/function studies and for the preparation of polyclonal and monoclonal antibodies. Polypeptides useful as immunogenic components for preparing antibodies or as targets for binding agent studies are at least five or more residues in length. Preferably, the polypeptides comprise at least about 12, more preferably at least about 20, and most preferably at least about 30 or more residues. Methods for obtaining these polypeptides are well known and are explained in *Immunochemical Methods in Cell and Molecular Biology*, 1987 (Mayer and Waler, eds; Academic Press, London); Scopes, 1987, *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.) and *Handbook of Experimental Immunology*, 1986, Volumes I-IV (Weir and Blackwell, eds.).

Having isolated one member of a binding partner of a specific interaction, methods exist for isolating the counter-partner. See, e.g., Gearing *et al.*, 1989, *EMBO J.* **8**:3667-3676. Many methods of screening for binding activity are known by those skilled in the art and may be used to practice the invention. For example, an expression library can be screened for specific binding to the protein, e.g., by cell sorting, or other screening to detect subpopulations which express such a binding component. See, e.g., Ho *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* **90**:11267-11271. Alternatively, a panning method may be used. See, e.g., Seed and Aruffo, 1987, *Proc. Natl. Acad. Sci. USA* **84**:3365-3369. A two-hybrid selection system may also be applied making appropriate constructs with the available protein sequences. See, e.g., Fields and Song, 1989, *Nature* **340**:245-246. Several methods of automated assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period of time.

Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence similarity with an amino acid sequence of a SEQ ID NO: 2, 4, 6, 8 or 10. Variants exhibiting substitutions, e.g., 20 or fewer, preferably 10 or fewer, and more preferably 5 or fewer substitutions, are encompassed. Where the substitutions are conservative substitutions, the variants will share immunogenic or antigenic similarity or cross-reactivity with a corresponding natural sequence protein. Natural variants include individual, allelic, polymorphic, strain, or species variants.

Amino acid sequence similarity, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. This changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Homologous amino acid sequences include natural allelic and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 50-100% similarity (if gaps can be introduced), to 75-100% similarity (if conservative substitutions are included) with the amino acid sequence of the relevant protein. Identity measures will be at least about 50%, generally at least 60%, more generally at least 65%, usually at least 70%, more usually at least 75%, preferably at least 80%, and more preferably at least 80%, and in particularly preferred embodiments, at least 85% or more. See also Needleham *et al.*, 1970, *J. Mol. Biol.* 48:443-453; Sankoff *et al.*, 1983, *Time Warps, String Edits, and*

Macromolecules: The Theory and Practice of Sequence Comparison Chapter One, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group (GCG), Madison, WI.

Nucleic acids encoding the corresponding proteins will typically hybridize to SEQ ID NO: 1, 3, 5, 7 or 9 under stringent conditions. For example, nucleic acids encoding the respective proteins will typically hybridize to the nucleic acid of SEQ ID NO: 1, 3, 5, 7 or 9 under stringent hybridization conditions, while providing few false positive hybridization signals. Generally, stringent conditions are selected to be about 10° C lower than the thermal melting point (Tm) for the sequence being hybridized to at a defined ionic strength and pH. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration in wash is about 0.02 molar at pH 7 and the temperature is at least about 50° C. Other factors may significantly affect the stringency of hybridization, including, among others, base composition and size of the complementary strands, the presence of organic solvents such as formamide, and the extent of base mismatching. A preferred embodiment will include nucleic acids that will bind to disclosed sequences in 50% formamide and 20-50 mM NaCl at 42° C.

An isolated nucleic acid can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having highly similar physiological, immunogenic, or antigenic activity.

Modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. Such mutant protein derivatives include predetermined or site-specific mutations of the respective protein or its fragments. "Mutant protein" encompasses a polypeptide otherwise falling within the homology definition of the proteins as set forth above, but having an amino acid sequence which differs from that of the protein as found in nature, whether by way of deletion, substitution, or insertion. In particular, "site specific mutant protein" generally includes proteins having significant similarity with a protein having a sequence of SEQ ID NO: 2, 4, 6, 8 or 10. Generally, the variant will share many physicochemical and biological

activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most or all of the disclosed sequence.

Glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. Particularly preferred means for accomplishing this are by exposing the polypeptide to glycosylating enzymes derived from cells that normally provide such processing, e.g., mammalian glycosylation enzymes. Deglycosylation enzymes are also contemplated. Also embraced are versions of the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine, or other moieties, including ribosyl groups or cross-linking reagents. Also, proteins comprising substitutions are encompassed, which should retain substantial immunogenicity, to produce antibodies that recognize a protein of SEQ ID NO: 2, 4, 6, 8 or 10. Typically, these proteins will contain less than 20 residue substitutions from the disclosed sequence, more typically less than 10 substitutions, preferably less than 5, and more preferably less than three. Alternatively, proteins that begin and end at structural domains will usually retain antigenicity and cross immunogenicity.

A major group of derivatives are covalent conjugates of the proteins described herein or fragments thereof with other proteins or polypeptides. These derivatives can be synthesized in recombinant culture such as N- or C-terminal fusions or by the use of agents known in the art for their usefulness in cross-linking proteins through reactive side groups. Preferred protein derivatization sites with cross-linking agents are at free amino groups, carbohydrate moieties, and cysteine residues.

Fusion polypeptides between these proteins and other homologous or heterologous proteins are also provided. Heterologous polypeptides may be fusions between different surface markers, resulting in, e.g., a hybrid protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a protein, e.g., a receptor-binding segment, so that the presence or location of the fused protein may be easily determined. See, e.g., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial ß-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, and yeast alpha mating factor. See, e.g., Godowski *et al.*, 1988, *Science* **241**:812-816.

Such polypeptides may also have amino acid residues that have been chemically modified by phosphorylation, sulfonation, biotinylation, or the addition or removal of other moieties, particularly those that have molecular shapes similar to phosphate groups. In some embodiments, the modifications will be useful labeling reagents, or serve as purification targets, e.g., affinity ligands.

This invention also contemplates the use of derivatives of these proteins other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties. These derivatives generally fall into the three classes: (1) salts, (2) side chain and terminal residue covalent modifications, and (3) adsorption complexes, for example with cell membranes. Such covalent or aggregative derivatives are useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of ligands or other binding ligands. For example, a protein antigen can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated Sepharose, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of antibodies. The proteins can also be labeled with a detectable group, e.g., radioiodinated by the chloramine T procedure, covalently bound to rare earth chelates, or conjugated to another fluorescent moiety for use in diagnostic assays. Purification of these proteins may be accomplished by immobilized antibodies.

Antibodies

The immunogenic components of this invention, as discribed above, are useful as antigens for preparing antibodies by standard methods. Such immunogenic components can be produced by proteolytic cleavage of larger polypeptides or by chemical synthesis or recombinant technology and are thus not limited by proteolytic cleavage sites. Preferably, smaller immunogenic components will first be rendered more immunogenic by cross-linking or by coupling to an immunogenic carrier molecule (i.e., a macromolecule having the property of independently eliciting an immunological response in a host animal, to which the immunogenic components of the invention can be covalently linked). Cross-linking or conjugation to a carrier molecule may be required because small polypeptide fragments sometimes act as haptens (molecules which are capable of specifically binding to an antibody but incapable of eliciting antibody production, i.e., they are not immunogenic). Conjugation of such fragments to an

immunogenic carrier molecule renders them immunogenic through what is commonly known as the "carrier effect".

Antibodies according to the present invention include polyclonal and monoclonal antibodies. The antibodies may be elicited in an animal host by immunization with immunogenic components of the invention or may be formed by *in vitro* immunization (sensitization) of immune cells. The immunogenic components used to elicit the production of antibodies may be isolated from human cells (e.g., human monocytes) or chemically synthesized. The antibodies may also be produced in recombinant systems programmed with appropriate antibody-encoding DNA. Alternatively, the antibodies may be constructed by biochemical reconstitution of purified heavy and light chains.

The antibodies of this invention can be purified by standard methods, including but not limited to preparative disc-gel electrophoresis, isoelectric focusing, HPLC, reversed-phase HPLC, gel filtration, ion exchange and partition chromatography, and countercurrent distribution. Purification methods for antibodies are disclosed, e.g., in *The Art of Antibody Purification*, 1989, Amicon Division, W.R. Grace & Co. General protein purification methods are described in *Protein Purification: Principles and Practice*, R.K. Scopes, Ed., 1987, Springer-Verlag, New York, NY.

Suitable adjuvants for the vaccination of animals include but are not limited to Adjuvant 65 (containing peanut oil, mannide monooleate and aluminum monostearate); Freund's complete or incomplete adjuvant; mineral gels such as aluminum hydroxide, aluminum phosphate and alum; surfactants such as hexadecylamine, octadecylamine, lysolecithin, dimethyldioctadecyl-ammonium bromide, N,N-dioctadecyl-N',N'-bis(2-hydroxymethyl) propane-diamine, methoxyhexadecylglycerol and pluronic polyols; polyanions such as pyran, dextran sulfate, poly IC, polyacrylic acid and carbopol; peptides such as muramyl dipeptide, dimethylglycine and tuftsin; and oil emulsions. The immunogenic components could also be administered following incorporation into liposomes or other microcarriers. Information concerning adjuvants and various aspects of immunoassays are disclosed, e.g., in the series by P. Tijssen, 1987, *Practice and Theory of Enzyme Immunoassays*, 3rd Edition, Elsevier, New York.

Serum produced from animals thus immunized can be used directly. Alternatively, the IgG fraction can be separated from the serum using standard methods such as plasmaphoresis or adsorption chromatography using IgG specific adsorbents such as immobilized Protein A.

Hybridomas of the invention used to make monoclonal antibodies against the immunogenic components of the invention are produced by well-known techniques.

Usually, the process involves the fusion of an immortalizing cell line with a B-lymphocyte that produces the desired antibody. Alternatively, non-fusion techniques for generating immortal antibody-producing cell lines are possible, and come within the purview of the present invention, e.g., virally-induced transformation, Casali *et al.*, 1986, *Science* **234**:476. Immortalizing cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine, and human origin. Most frequently, rat or mouse myeloma cell lines are employed as a matter of convenience and availability.

Techniques for obtaining the appropriate lymphocytes from mammals injected with the immunogenic components are well known. Generally, peripheral blood lymphocytes (PBLs) are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. A host animal is injected with repeated dosages of a preferably purified immunogenic component, and the animal is permitted to generate the desired antibody-producing cells before these are harvested for fusion with the immortalizing cell line. Techniques for fusion are also well known in the art, and in general involve mixing the cells with a fusing agent, such as polyethylene glycol.

Hybridomas are selected by standard procedures, such as HAT (hypoxanthine-aminopterin-thymidine) selection. From among these hybridomas, those secreting the desired antibody are selected by assaying their culture medium by standard immunoassays, such as Western blotting, ELISA (enzyme-linked immunosorbent assay), RIA (radioimmunoassay), or the like. Antibodies are recovered from the medium using standard protein purification techniques, Tijssen, 1985, *Practice and Theory of Enzyme Immunoassays*, Elsevier, Amsterdam.

Many references are available for guidance in applying any of the above techniques: Kohler *et al.*, 1980, *Hybridoma Techniques*, Cold Spring Harbor Laboratory, New York; Tijssen, 1985, *Practice and Theory of Enzyme Immunoassays*, Elsevier, Amsterdam; Campbell, 1984, *Monoclonal Antibody Technology*, Elsevier, Amsterdam; Hurrell, 1982, *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Boca Raton, FL. Monoclonal antibodies can also be produced using well known phage library systems.

The use and generation of antibody fragments is also well known, e.g., Fab fragments: Tijssen, 1985, *Practice and Theory of Enzyme Immunoassays*, Elsevier, Amsterdam; Fv fragments: Hochman *et al.*, 1973, *Biochemistry* **12**:1130; Sharon *et al.*, 1976, *Biochemistry* **15**:1591; Ehrlich *et al.*, U.S. Patent No. 4,355,023; and antibody half

molecules: Auditore-Hargreaves, U.S. Patent No. 4,470,925. These also may be useful in immunoassays.

These antibodies, whether polyclonal or monoclonal, can be used, e.g., in an immobilized form bound to a solid support by well known methods, to isolate and purify the immunogenic components by immunoaffinity chromatography. The antibodies are useful as probes to distinguish tissue and cell type distribution. The antibodies may be used to screen expression libraries for particular expression products. Usually the antibodies used in such a procedure will be labeled with a moiety allowing easy detection of presence of antigen by antibody binding. Antibodies to proteins may be used for the analysis or, or identification of specific cell population components which express the respective protein. By assaying the expression products of cells expressing the proteins described herein it is possible to diagnose disease, e.g., immune-compromised conditions, monocyte depleted conditions, or overproduction of monocytes. Antibodies raised against the proteins will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens. The present invention encompasses antibodies that specifically recognize monocyte-derived immunogenic components. Such antibodies can be used conventionally, e.g., as reagents for purification of monocyte cell components, or in diagnostic applications.

Diagnostic Applications

The invention encompasses compositions, methods, and kits useful in clinical settings for the qualitative or quantitative diagnosis, i.e., detection of specific components in a biological sample. These applications utilize nucleic acids, peptides/polypeptides, or antibodies specific for the components described herein. Both antibody-based and nucleic acid-based diagnostic methods, including PCR-based diagnostic methods are contemplated. Detection of the level of monocyte cells present in a sample is important for diagnosis of certain aberrant disease conditions. For example, an increase in the number of monocytes in a tissue or the lymph system can be indicative of the presence of a monocyte hyperplasia, tissue or graft rejection, or inflammation. A low monocyte population can indicate an abnormal reaction to, e.g., a bacterial or viral infection, which may require an appropriate treatment to normalize the monocyte response.

Both the naturally occurring and the recombinant form of the proteins of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins.

In nucleic-acid-type diagnostic methods, the sample to be analyzed may be contacted directed with the nucleic acid probes. Probes include oligonucleotides at least 12 nucleotides, preferably at least 18, and most preferably 20-35 or more nucleotides in length. Alternatively, the sample may be treated to extract the nucleic acids contained therein. It will be understood that the particular method used to extract DNA will depend on the nature of the biological sample. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques, or, the nucleic acid sample may be immobilized on an appropriate solid matrix without size separation or used for PCR.

Kits suitable for antibody-based diagnostic applications typically include one or more of the following components:

(i) Antibodies: The antibodies may be pre-labeled; alternatively, the antibody may be unlabelled and the ingredients for labeling may be included in the kit in separate containers, or a secondary, labeled antibody is provided; and

(ii) Reaction components: The kit may also contain other suitably packaged reagents and materials needed for the particular immunoassay protocol, including solid-phase matrices, if applicable, and standards.

Kits suitable for nucleic acid-based diagnostic applications typically include the following components:

(i) *Probe DNA*: The probe DNA may be pre-labeled; alternatively, the probe DNA may be unlabelled and the ingredients for labeling may be included in the kit in separate containers; and

(ii) *Hybridization reagents*: The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, including solid-phase matrices, if applicable, and standards.

PCR based diagnostic kits are also contemplated and are encompassed by the invention.

The kits referred to above may include instructions for conducting the test. Furthermore, in preferred embodiments, the diagnostic kits are adaptable to high-throughput and/or automated operation.

Therapeutic Applications

The invention also provides reagents that may exhibit significant therapeutic value. The proteins (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to the

proteins, may be useful in the treatment of conditions associated with abnormal physiology or development. For example, a disease or disorder associated with abnormal expression or abnormal signaling by a monocyte, e.g., as an antigen presenting cell, is a target for an agonist or antagonist of the protein. The proteins likely play a role in regulation or development of hematopoietic cells, e.g., lymphoid cells, which affect immunological responses, e.g., antigen presentation and the resulting effector functions.

Other abnormal developmental conditions are known in cell types shown to possess monocyte protein mRNA by northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, NJ; and Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, NY. Developmental or functional abnormalities, e.g., of the immune system, cause significant medical abnormalities and conditions that may be susceptible to prevention or treatment using compositions provided herein.

Recombinant monocyte-derived proteins or antibodies of the invention may be purified and then administered to a patient. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous stabilizers and excipients. In particular, these may be useful in a vaccine context, where the antigen is combined with one of these therapeutic versions of agonists or antagonists. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using antibodies or receptor or fragments thereof can identify compounds having binding affinity to these monocyte-derived proteins, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound blocks or antagonizes the activity of the protein. Likewise, a compound having intrinsic stimulating activity might activate the cell through the protein and is thus an agonist. This invention further contemplates the therapeutic use of antibodies to the proteins as antagonists.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal

testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, NJ. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous administration.

The proteins, antagonists, and agonists could be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds.) (1990) Goodman and Gilman's: The Pharmacological Bases of Therapeutics (8th ed.) Pergamon Press; and (1990) Remington's Pharmaceutical Sciences (17th ed.) Mack Publishing Co., Easton, PA; Avis, et al. (eds.) (1993) Pharmaceutical Dosage Forms: Parenteral Medications Dekker, NY; Lieberman, et al. (eds.) (1990) Pharmaceutical Dosgae Forms: Tablets Dekker, NY; and Lieberman, et al. (eds.) (1990) Pharmaceutical Dosage Forms: Disperse Systems Dekker, NY. The therapy of this invention may be combined with or used in association with other chemotherapeutic or chemopreventive agents.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1. An isolated polypeptide comprising an amino acid sequence derived from SEQ ID NO: 2, 4, 6, 8 or 10.

2. The polypeptide of claim 1 comprising the amino acid sequence of the mature protein.

3. An isolated nucleic acid comprising a nucleotide sequence encoding an amino acid sequence derived from SEQ ID NO: 2, 4, 6, 8 or 10.

4. The nucleic acid of claim 3 wherein the nucleotide sequence encodes the mature protein.

5. The nucleic acid of claim 4 comprising the nucleotide sequence shown in SEQ ID: NO 1, 3, 5, 7 or 9.

6. A fusion protein comprising the polypeptide of claim 1.

7. A binding compound which specifically binds to the polypeptide of claim 1.

8. The binding compound of claim 7 which is an antibody or antibody fragment.

9. The binding compound of claim 8 wherein the anitbody is a monoclonal antibody.

10. An expression vector comprising the nucleic acid of claim 3.

11. A host cell comprising the vector of claim 10.

12. A process for recombinatly producing a polypeptide comprising culturing the host cell of claim 11 under conditions in which the polypeptide is expressed.

13. A method for detecting a specific nucleic acid sequence in a sample, said method comprising the steps of:

(i) contacting a sample suspected to contain a specific nucleic acid sequence with a probe comprising a nucleic acid sequence comprising at least 8 consecutive nucleotides

selected from SEQ ID NO: 1, 3, 5, 7, or 9 under conditions in which a hybrid can form between said probe and the specific nucleic acid in said sample; and

(ii) detecting any hybrid formed in step (i),

wherein detection of said hybrid indicates the presence of the specific nucleic acid sequence in said sample.

14. The method of claim 13 further comprising amplifying said specific sequence in said sample prior to said detecting step.

15. A method for detecting a specific antigenic component in a sample, said method comprising the steps of:

(i) contacting a sample suspected to contain a specific antigenic component encoded by an amino acid sequence derived from SEQ ID NO: 2, 4, 6, 8, or 10 with an antibody specific for said component, under conditions in which a stable antigen-antibody complex can form between said antibody and said antigenic component in said sample; and

(ii) detecting any antigen-antibody complex formed in step (i),

wherein detection of an antigen-antibody complex indicates the presence of said antigenic component in said sample.

16. A method of screening for candidate therapeutic agents comprising:

selecting as a target sequence a polypeptide having an amino acid sequence derived from SEQ ID NO: 2, 4, 6, 8 or 10;

contacting a test compound with said target sequence; and

selecting as candidate therapeutic agent those test compounds which bind to the target sequence.

MONOCYTE-DERIVED NUCLEIC ACIDS AND RELATED COMPOSITIONS AND METHODS

Abstract Of The Disclosure

Nucleic acids encoding various monocyte-derived proteins and related compositions, including purified proteins and specific antibodies are described. Methods of using such composition are also provided.

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

Attorney's Docket No. **SF0977X**

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my/our name;

I believe I am the original, first sole inventor (if only one name is listed below) or an original, first
and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

"MONOCYTE-DERIVED NUCLEIC ACIDS AND RELATED COMPOSITIONS AND METHODS"

the specification of which

[X] is attached hereto.

[ ] was filed on _____ as Application Serial No. _____

and was amended on _____ (if applicable).

[ ] was filed on _____ as PCT International Application No. _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s):			Priority Claimed
(Number)	(Country)	(Day/Month/Year Filed)	Yes or No

I hereby claim the benefit under Title 35, United States Code, §119(e) of any United States provisional application(s) listed below:

(Application Number)	(Filing Date)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

09/223,919	December 31, 1998	
(Application Serial No.)	(Filing Date)	(Status – patented, pending, abandoned)
09/224,604	December 31, 1998	
(Application Serial No.)	(Filing Date)	(Status – patented, pending, abandoned)

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office
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I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature of First Inventor	**Signature of Second Inventor**	**Signature of Third Inventor**
		CHALUS
Date 4/01/2000	Date 21/12/99	Date 21/12/99
Signature of ~~Fourth~~ Inventor		
Date 17/12/1999		

Rev. 2/96 JHB

CASE NO. SF0977X

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Elizabeth Bates, *et al.*

Serial No.: 09/869,388

Filed: December 29, 1999

Atty. Docket No.: SF0977X

For: Monocyte Derived Nucleic Acids and Related Compositions and Methods

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

ASSOCIATE POWER OF ATTORNEY

Pursuant to 37 C.F.R. § 1.34(b) Thomas Triolo, Registration No. 48,001 is hereby appointed Associate Power of Attorney for the above-identified patent application.

Respectfully submitted,

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Fournier, Nathalie
Chalus, Lionel
Garrone, Pierre

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Ser Tyr Leu Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met
         15                  20                  25
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```
ggt ggc tct gtg gaa atc ccc ttc tcc ttc tat tac ccc tgg gag tta       312
Gly Gly Ser Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu
     30                  35                  40

gcc aca gct ccc gac gtg aga ata tcc tgg aga cgg ggc cac ttc cac       360
Ala Thr Ala Pro Asp Val Arg Ile Ser Trp Arg Arg Gly His Phe His
 45                  50                  55                  60

ggg cag tcc ttc tac agc aca agg ccg cct tcc att cac aag gat tat       408
Gly Gln Ser Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr
                 65                  70                  75

gtg aac cgg ctc ttt ctg aac tgg aca gag ggt cag aag agc ggc ttc       456
Val Asn Arg Leu Phe Leu Asn Trp Thr Glu Gly Gln Lys Ser Gly Phe
             80                  85                  90

ctc agg atc tcc aac ctg cag aag cag gac cag tct gtg tat ttc tgc       504
Leu Arg Ile Ser Asn Leu Gln Lys Gln Asp Gln Ser Val Tyr Phe Cys
         95                  100                 105

cga gtt gag ctg gac aca cgg agc tca ggg agg cag cag tgg cag tcc       552
Arg Val Glu Leu Asp Thr Arg Ser Ser Gly Arg Gln Gln Trp Gln Ser
    110                 115                 120

atc gag ggg acc aaa ctc tcc atc acc cag ggt cag cag cgg act aaa       600
Ile Glu Gly Thr Lys Leu Ser Ile Thr Gln Gly Gln Gln Arg Thr Lys
125                 130                 135                 140

gcc aca acc cca gcc agg gaa ccc ttc caa aac aca gag gag cca tat       648
Ala Thr Thr Pro Ala Arg Glu Pro Phe Gln Asn Thr Glu Glu Pro Tyr
                145                 150                 155

gag aat atc agg aat gaa gga caa aat aca gat ccc aag cta aat ccc       696
Glu Asn Ile Arg Asn Glu Gly Gln Asn Thr Asp Pro Lys Leu Asn Pro
            160                 165                 170

aag gat gac ggc atc gtc tat gct tcc ctt gcc ctc tcc agc tcc acc       744
Lys Asp Asp Gly Ile Val Tyr Ala Ser Leu Ala Leu Ser Ser Ser Thr
        175                 180                 185

tca ccc aga gca cct ccc agc cac cgt ccc ctc aag agc ccc cag aac       792
Ser Pro Arg Ala Pro Pro Ser His Arg Pro Leu Lys Ser Pro Gln Asn
    190                 195                 200

gag acc ctg tac tct gtc tta aag gcc taaccaatgg acagccctct             839
Glu Thr Leu Tyr Ser Val Leu Lys Ala
205                 210

caagactgaa tggtgaggcc aggtacagtg gcgcacacct gtaatcccag ctactctgaa     899

gcctgaggca gaatcaagtg agcccaggag ttcagggcca gctt                      943
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<210> 4
<211> 230
<212> PRT
<213> homo sapiens

<400> 4

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Met Gly Arg Pro Leu Leu Leu Pro Leu Leu Pro Leu Leu Leu Pro Pro
-17     -15                 -10                  -5

Ala Phe Leu Gln Pro Ser Gly Ser Thr Gly Ser Gly Pro Ser Tyr Leu
      1               5                  10                  15

Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met Gly Gly Ser
                 20                  25                  30

Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu Ala Thr Ala
             35                  40                  45

Pro Asp Val Arg Ile Ser Trp Arg Arg Gly His Phe His Gly Gln Ser
         50                  55                  60

Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr Val Asn Arg
     65                  70                  75

Leu Phe Leu Asn Trp Thr Glu Gly Gln Lys Ser Gly Phe Leu Arg Ile
 80                  85                  90                  95

Ser Asn Leu Gln Lys Gln Asp Gln Ser Val Tyr Phe Cys Arg Val Glu
                100                 105                 110

Leu Asp Thr Arg Ser Ser Gly Arg Gln Gln Trp Gln Ser Ile Glu Gly
            115                 120                 125

Thr Lys Leu Ser Ile Thr Gln Gly Gln Gln Arg Thr Lys Ala Thr Thr
        130                 135                 140

Pro Ala Arg Glu Pro Phe Gln Asn Thr Glu Glu Pro Tyr Glu Asn Ile
    145                 150                 155

Arg Asn Glu Gly Gln Asn Thr Asp Pro Lys Leu Asn Pro Lys Asp Asp
160                 165                 170                 175

Gly Ile Val Tyr Ala Ser Leu Ala Leu Ser Ser Ser Thr Ser Pro Arg
                180                 185                 190

Ala Pro Pro Ser His Arg Pro Leu Lys Ser Pro Gln Asn Glu Thr Leu
            195                 200                 205

Tyr Ser Val Leu Lys Ala
        210
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<210> 5
<211> 1450
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (386)..(1066)

<220>
<221> sig_peptide
<222> (386)..(436)

<220>
<221> mat_peptide
<222> (437)..(1066)

<400> 5
ccacgcgtcc ggcttctttg ggggtgaaga gattggggag gaatctccac ccctgggagg 60

cagaagccag gcatagcgcg ctggctagga ctccagtacc gtgaagggag gcagtgagag 120

cagacatctg tgcctcattc ctgatctcaa ggggaaagca agaacaaggg aggcttcctc 180

aggatctcga acctgcggaa ggaggaccag tctgtgtact tctgccaagt ccagctggac 240

atacagatca gggaggctgt cgtggcagtc catcaagggg acccacctca ccatcaccca 300

ggccctcagg cagcccctcc acagggcccc tctcctgcct ggacagctct gctggtctcc 360

ccgtcccctg gagaagaaca aggcc atg ggt cgg ccc ctg ctg ctg ccc ctg 412
Met Gly Arg Pro Leu Leu Leu Pro Leu
-17 -15 -10

ctg ctc ctg ctg cag ccg cca gca ttt ctg cag cct ggt ggc tcc aca 460
Leu Leu Leu Leu Gln Pro Pro Ala Phe Leu Gln Pro Gly Gly Ser Thr
-5 1 5

gga tct ggt cca agc tac ctt tat ggg gtc act caa cca aaa cac ctc 508
Gly Ser Gly Pro Ser Tyr Leu Tyr Gly Val Thr Gln Pro Lys His Leu
10 15 20

tca gcc tcc atg ggt ggc tct gtg gaa atc ccc ttc tcc ttc tat tac 556
Ser Ala Ser Met Gly Gly Ser Val Glu Ile Pro Phe Ser Phe Tyr Tyr
25 30 35 40

ccc tgg gag tta gcc ata gtt ccc aac gtg aga ata tcc tgg aga cgg 604
Pro Trp Glu Leu Ala Ile Val Pro Asn Val Arg Ile Ser Trp Arg Arg
45 50 55

ggc cac ttc cac ggg cag tcc ttc tac agc aca agg ccg cct tcc att 652
Gly His Phe His Gly Gln Ser Phe Tyr Ser Thr Arg Pro Pro Ser Ile
60 65 70

cac aag gat tat gtg aac cgg ctc ttt ctg aac tgg aca gag ggt cag 700
His Lys Asp Tyr Val Asn Arg Leu Phe Leu Asn Trp Thr Glu Gly Gln
75 80 85

gag agc ggc ttc ctc agg atc tca aac ctg cgg aag gag gac cag tct 748
Glu Ser Gly Phe Leu Arg Ile Ser Asn Leu Arg Lys Glu Asp Gln Ser
90 95 100

gtg tat ttc tgc cga gtc gag ctg gac acc cgg aga tca ggg agg cag 796
Val Tyr Phe Cys Arg Val Glu Leu Asp Thr Arg Arg Ser Gly Arg Gln
105 110 115 120

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cag ttg cag tcc atc aag ggg acc aaa ctc acc atc acc cag gct gtc       844
Gln Leu Gln Ser Ile Lys Gly Thr Lys Leu Thr Ile Thr Gln Ala Val
                125                 130                 135

aca acc acc acc acc tgg agg ccc agc agc aca acc acc ata gcc ggc       892
Thr Thr Thr Thr Thr Trp Arg Pro Ser Ser Thr Thr Thr Ile Ala Gly
            140                 145                 150

ctc agg gtc aca gaa agc aaa ggg cac tca gaa tca tgg cac cta agt       940
Leu Arg Val Thr Glu Ser Lys Gly His Ser Glu Ser Trp His Leu Ser
        155                 160                 165

ctg gac act gcc atc agg gtt gca ttg gct gtc gct gtg ctc aaa act       988
Leu Asp Thr Ala Ile Arg Val Ala Leu Ala Val Ala Val Leu Lys Thr
    170                 175                 180

gtc att ttg gga ctg ctg tgc ctc ctc ctc ctg tgg tgg agg aga agg      1036
Val Ile Leu Gly Leu Leu Cys Leu Leu Leu Leu Trp Trp Arg Arg Arg
185                 190                 195                 200

aaa ggt agc agg gcg cca agc agt gac ttc tgaccaacag agtgtgggga       1086
Lys Gly Ser Arg Ala Pro Ser Ser Asp Phe
                205                 210

gaagggatgt gtattagccc cggaggacgt gatgtgagac ccgcttgtga gtcctccaca   1146

ctcgttcccc attggcaaga tacatggaga gcaccctgag gacctttaaa aggcaaagcc   1206

gcaaggcaga aggaggctgg gtccctgaat caccgactgg aggagagtta cctacaagag   1266

ccttcatcca ggagcatcca cactgcaatg atataggaat gaggtctgaa ctccactgaa   1326

ttaaaccact ggcatttggg ggctgtttat tatagcagtg caaagagttc ctttatcctc   1386

cccaaggatg gaaaaataca atttattttg cttaccataa aaaaaaaaaa aaaaaaaaaa   1446

aaaa                                                                1450


<210> 6
<211> 227
<212> PRT
<213> homo sapiens

<400> 6
Met Gly Arg Pro Leu Leu Leu Pro Leu Leu Leu Leu Leu Gln Pro Pro
-17     -15                 -10                 -5

Ala Phe Leu Gln Pro Gly Gly Ser Thr Gly Ser Gly Pro Ser Tyr Leu
      1               5                  10                  15

Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met Gly Gly Ser
                20                  25                  30

Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu Ala Ile Val
            35                  40                  45
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Pro Asn Val Arg Ile Ser Trp Arg Arg Gly His Phe His Gly Gln Ser
          50                  55                  60

Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr Val Asn Arg
      65                  70                  75

Leu Phe Leu Asn Trp Thr Glu Gly Gln Glu Ser Gly Phe Leu Arg Ile
 80                  85                  90                  95

Ser Asn Leu Arg Lys Glu Asp Gln Ser Val Tyr Phe Cys Arg Val Glu
                100                 105                 110

Leu Asp Thr Arg Arg Ser Gly Arg Gln Gln Leu Gln Ser Ile Lys Gly
            115                 120                 125

Thr Lys Leu Thr Ile Thr Gln Ala Val Thr Thr Thr Thr Thr Trp Arg
        130                 135                 140

Pro Ser Ser Thr Thr Thr Ile Ala Gly Leu Arg Val Thr Glu Ser Lys
    145                 150                 155

Gly His Ser Glu Ser Trp His Leu Ser Leu Asp Thr Ala Ile Arg Val
160                 165                 170                 175

Ala Leu Ala Val Ala Val Leu Lys Thr Val Ile Leu Gly Leu Leu Cys
                180                 185                 190

Leu Leu Leu Leu Trp Trp Arg Arg Arg Lys Gly Ser Arg Ala Pro Ser
            195                 200                 205

Ser Asp Phe
        210
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<210> 7
<211> 909
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (130)..(657)

<220>
<221> sig_peptide
<222> (130)..(180)

<220>
<221> mat_peptide
<222> (181)..(654)

<400> 7
acagccctct tcggagcctc agcccggctc tcctcactca cctcaacccc caggcggccc 60

ctccacaggg cccctctcct gcctggacgg ctctgctggt ctccccgtcc cctggagaag 120

aacaaggcc atg ggt cgg ccc ctg ctg ctg ccc cta ctg ccc ctg ctg ctg 171

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                Met Gly Arg Pro Leu Leu Leu Pro Leu Leu Pro Leu Leu Leu
                      -15                 -10                  -5

ccg cca gca ttt ctg cag cct agt ggc tcc aca gga tct ggt cca agc   219
Pro Pro Ala Phe Leu Gln Pro Ser Gly Ser Thr Gly Ser Gly Pro Ser
         -1   1               5                  10

tac ctt tat ggg gtc act caa cca aaa cac ctc tca gcc tcc atg ggt   267
Tyr Leu Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met Gly
     15                  20                  25

ggc tct gtg gaa atc ccc ttc tcc ttc tat tac ccc tgg gag tta gcc   315
Gly Ser Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu Ala
 30                  35                  40                  45

aca gct ccc gac gtg aga ata tcc tgg aga cgg ggc cac ttc cac ggg   363
Thr Ala Pro Asp Val Arg Ile Ser Trp Arg Arg Gly His Phe His Gly
                50                  55                  60

cag tcc ttc tac agc aca agg ccg cct tcc att cac aag gat tat gtg   411
Gln Ser Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr Val
             65                  70                  75

aac cgg ctc ttt ctg aac tgg aca gag ggt cag aag agc ggc ttc ctc   459
Asn Arg Leu Phe Leu Asn Trp Thr Glu Gly Gln Lys Ser Gly Phe Leu
         80                  85                  90

agg atc tcc aac ctg cag aag cag gac cag tct gtg tat ttc tgc cga   507
Arg Ile Ser Asn Leu Gln Lys Gln Asp Gln Ser Val Tyr Phe Cys Arg
     95                  100                 105

gtt gag ctg gac aca cgg agc tca ggg agg cag cag tgg cag tcc atc   555
Val Glu Leu Asp Thr Arg Ser Ser Gly Arg Gln Gln Trp Gln Ser Ile
110                 115                 120                 125

gag ggg acc aaa ctc tcc atc acc cag ggg aac cct tcc aaa aca cag   603
Glu Gly Thr Lys Leu Ser Ile Thr Gln Gly Asn Pro Ser Lys Thr Gln
                130                 135                 140

agg agc cat atg aga ata tca gga atg aag gac aaa ata cag atc cca   651
Arg Ser His Met Arg Ile Ser Gly Met Lys Asp Lys Ile Gln Ile Pro
            145                 150                 155

agc taa atcccaagga tgacggcatc gtctatgctt cccttgccct ctccagctcc    707
Ser

acctcaccca gagcacctcc cagccaccgt cccctcaaga gcccccagaa cgagaccctg 767

tactctgtct taaaggccta accaatggac agccctctca agactgaatg gtgaggccag 827

gtacagtggc gcacacctgt aatcccagct actctgaagc ctgaggcaga atcaagtgag 887

cccaggagtt cagggccagc tt                                          909
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<210> 8
<211> 175

<212> PRT
<213> homo sapiens

<400> 8
Met Gly Arg Pro Leu Leu Leu Pro Leu Leu Pro Leu Leu Leu
-15 -10 -5

Pro Pro Ala Phe Leu Gln Pro Ser Gly Ser Thr Gly Ser Gly Pro Ser
-1 1 5 10

Tyr Leu Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met Gly
15 20 25

Gly Ser Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu Ala
30 35 40 45

Thr Ala Pro Asp Val Arg Ile Ser Trp Arg Arg Gly His Phe His Gly
50 55 60

Gln Ser Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr Val
65 70 75

Asn Arg Leu Phe Leu Asn Trp Thr Glu Gly Gln Lys Ser Gly Phe Leu
80 85 90

Arg Ile Ser Asn Leu Gln Lys Gln Asp Gln Ser Val Tyr Phe Cys Arg
95 100 105

Val Glu Leu Asp Thr Arg Ser Ser Gly Arg Gln Gln Trp Gln Ser Ile
110 115 120 125

Glu Gly Thr Lys Leu Ser Ile Thr Gln Gly Asn Pro Ser Lys Thr Gln
130 135 140

Arg Ser His Met Arg Ile Ser Gly Met Lys Asp Lys Ile Gln Ile Pro
145 150 155

Ser

<210> 9
<211> 1459
<212> DNA
<213> homo sapiens

<220>
<221> CDS
<222> (309)..(989)

<220>
<221> sig_peptide
<222> (309)..(359)

<220>
<221> mat_peptide
<222> (360)..(986)

<400> 9

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ggcacgacgc cccatctcta ctaataaaaa aaaaaaaaaa ggatttgaag tcctggccgg 60

agcaattagg caagggataa aaaggcacct aaggcccttt tgcaataaga agccagatgg 120

ataaaggaag tgctggtcac cctggaggtg tactggtttg gggaaggtcc ccggccccca 180

cagccctctg gggagcctca ccctggctct ccccactcac ctcagccctc aggcagcccc 240

tccacaggac ccctctcctg cctggacagc tctgctggtc tccccgtccc ctggagaaga 300

acaaggcc atg ggt cgg ccc ctg ctg ctg ccc ctg ctg ctc ctg ctg cag  350
         Met Gly Arg Pro Leu Leu Leu Pro Leu Leu Leu Leu Leu Gln
                -15                 -10                  -5

ccg cca gca ttt ctg cag cct ggt ggc tcc aca gga tct ggt cca agc   398
Pro Pro Ala Phe Leu Gln Pro Gly Gly Ser Thr Gly Ser Gly Pro Ser
         -1   1               5                  10

tac ctt tat ggg gtc act caa cca aaa cac ctc tca gcc tcc atg ggt   446
Tyr Leu Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met Gly
     15                  20                  25

ggc tct gtg gaa atc ccc ttc tcc ttc tat tac ccc tgg gag tta gcc   494
Gly Ser Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu Ala
 30                  35                  40                  45

aca gct ccc gac gtg aga ata tcc tgg aga cgg ggc cac ttc cac ggg   542
Thr Ala Pro Asp Val Arg Ile Ser Trp Arg Arg Gly His Phe His Gly
                50                  55                  60

cag tcc ttc tac agc aca agg ccg cct tcc att cac aag gat tat gtg   590
Gln Ser Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr Val
             65                  70                  75

aac cgg ctc ttt ctg aac tgg aca gag ggt cag gag agc ggc ttc ctc   638
Asn Arg Leu Phe Leu Asn Trp Thr Glu Gly Gln Glu Ser Gly Phe Leu
         80                  85                  90

agg atc tca aac ctg cgg aag gag gac cag tct gtg tat ttc tgc cga   686
Arg Ile Ser Asn Leu Arg Lys Glu Asp Gln Ser Val Tyr Phe Cys Arg
     95                  100                 105

gtc gag ctg gac acc cgg aga tca ggg agg cag cag ttg cag tcc atc   734
Val Glu Leu Asp Thr Arg Arg Ser Gly Arg Gln Gln Leu Gln Ser Ile
110                 115                 120                 125

aag ggg acc aaa ctc acc atc acc cag gct gtc aca acc acc acc acc   782
Lys Gly Thr Lys Leu Thr Ile Thr Gln Ala Val Thr Thr Thr Thr Thr
                130                 135                 140

tgg agg ccc agc agc aca acc acc ata gcc ggc ctc agg gtc aca gaa   830
Trp Arg Pro Ser Ser Thr Thr Thr Ile Ala Gly Leu Arg Val Thr Glu
             145                 150                 155

agc aaa ggg cac tca gaa tca tgg cac cta agt ctg gac act gcc atc   878
Ser Lys Gly His Ser Glu Ser Trp His Leu Ser Leu Asp Thr Ala Ile
         160                 165                 170
```

```
agg gtt gca ttg gct gtc gct gtg ctc aaa act gtc att ttg gga ctg   926
Arg Val Ala Leu Ala Val Ala Val Leu Lys Thr Val Ile Leu Gly Leu
    175                 180                 185

ctg tgc ctc ctc ctg tgg tgg agg aga agg aaa ggt agc agg gcg cca   974
Leu Cys Leu Leu Leu Trp Trp Arg Arg Arg Lys Gly Ser Arg Ala Pro
190                 195                 200                 205

agc agt gac ttc tga ccaacagagt gtggggagaa gggatgtgta ttagccccgg   1029
Ser Ser Asp Phe

aggacgtgat gtgagacccg cttgtgagtc ctccacactc gttccccatt ggcaagatac 1089

atggagagca ccctgaggac ctttaaaagg caaagccgca aggcagaagg aggctgggtc 1149

cctgaatcac cgactggagg agagttacct acaagagcct tcatccagga gcatccacac 1209

tgcaatgata taggaatgag gtctgaactc cactgaatta aaccactggc atttgggggc 1269

tgttcattat agcagtgcaa agagttcctt tatcctcccc aaggatggaa aatacaattt 1329

attttgctta ccatacaccc cttttctcct cgtccacatt ttccaatctg tatggtggct 1389

gtcttctatg gcagaaggtt ttggggaata aatagcgtga aatgctgctg aaaaaaaaaa 1449

aaaaaaaaaa                                                        1459
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<210> 10
<211> 226
<212> PRT
<213> homo sapiens

<400> 10

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Met Gly Arg Pro Leu Leu Leu Pro Leu Leu Leu Leu Leu Gln
        -15                 -10                 -5

Pro Pro Ala Phe Leu Gln Pro Gly Gly Ser Thr Gly Ser Gly Pro Ser
         -1   1               5                  10

Tyr Leu Tyr Gly Val Thr Gln Pro Lys His Leu Ser Ala Ser Met Gly
     15                  20                  25

Gly Ser Val Glu Ile Pro Phe Ser Phe Tyr Tyr Pro Trp Glu Leu Ala
 30                  35                  40                  45

Thr Ala Pro Asp Val Arg Ile Ser Trp Arg Arg Gly His Phe His Gly
                50                  55                  60

Gln Ser Phe Tyr Ser Thr Arg Pro Pro Ser Ile His Lys Asp Tyr Val
             65                  70                  75

Asn Arg Leu Phe Leu Asn Trp Thr Glu Gly Gln Glu Ser Gly Phe Leu
         80                  85                  90
```

Arg Ile Ser Asn Leu Arg Lys Glu Asp Gln Ser Val Tyr Phe Cys Arg
95 100 105

Val Glu Leu Asp Thr Arg Arg Ser Gly Arg Gln Gln Leu Gln Ser Ile
110 115 120 125

Lys Gly Thr Lys Leu Thr Ile Thr Gln Ala Val Thr Thr Thr Thr Thr
130 135 140

Trp Arg Pro Ser Ser Thr Thr Thr Ile Ala Gly Leu Arg Val Thr Glu
145 150 155

Ser Lys Gly His Ser Glu Ser Trp His Leu Ser Leu Asp Thr Ala Ile
160 165 170

Arg Val Ala Leu Ala Val Ala Val Leu Lys Thr Val Ile Leu Gly Leu
175 180 185

Leu Cys Leu Leu Leu Trp Trp Arg Arg Arg Lys Gly Ser Arg Ala Pro
190 195 200 205

Ser Ser Asp Phe

<210> 11
<211> 21
<212> DNA
<213> homo sapiens

<400> 11
ACAGCCCTCT TCGGAGCCTC A 21

<210> 12
<211> 21
<212> DNA
<213> homo sapiens

<400> 12
AAGCTGGCCC TGAACTCCTG G 21

<210> 13
<211> 18
<212> DNA
<213> homo sapiens

<400> 13
CAAGGGATAA AAAGGCAC 18

<210> 14
<211> 18
<212> DNA
<213> homo sapiens

<400> 14
AACTCTCCTC CAGTCGGT 18